Invention Journal of Research Technology in Engineering & Management (IJRTEM)
ISSN: 2455-3689
www.ijrtem.com Volume 2 Issue 3 ǁ March. 2018 ǁ PP 36-43

Microbiological Profile and Quality Assessment of Unbranded Groundnut Oil Marketed at a Major City in Nigeria, Sub-Sahara Africa.

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ABSTRACT: *This work was conducted to assess the microbial profile and quality attributes of unbranded groundnut oil sold at Keffi. A total of 25 samples of unbranded groundnut oil were collected from different locations and subjected to microbial and quality assessment. The total viable bacteria count ranged from 2.1–7.2 × 10^5 cfu/ml, while the total faecal coliform count ranged from 2.2–6.2 × 10^5 cfu/ml. The Salmonella/Shigella count ranged from 1.4–4.2 × 10^5 cfu/ml and the fungal count ranged from 3.6 – 8.2 × 10^5 cfu/ml. The microbial isolates obtained were Mucor spp., Aspergillus flavus, Aspergillus niger, Rhizopus spp., Penicillium spp., Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus spp., Micrococcus spp., E. coli and Salmonella spp. Anti-biogram of the bacterial isolates revealed a varying level of resistance/susceptibility to the antibiotics tested. The result of mineral contents analysis showed that all samples had high detectable levels of Zn, Mn, Fe, Cu, Cd and Pb. These results indicated values that exceeded the maximum limits set by regulatory agencies, thereby making these oils unsafe for consumption. It can therefore be concluded that it is imperative for the manufacturers of these products to adopt good manufacturing practices and ensure proper quality assurance of their products.*

KEYWORDS: *Antibiotics, Groundnut oil, Microbiological profile, Keffi, Unbranded.*

I. INTRODUCTION

Groundnut (*Arachis hypogea*) or peanut belongs to the family of *Fabaceae* native from South and Central America [1]. Groundnuts often are enriched with health benefitting nutrients that are beneficial to human health. They are actually legumes but are the most frequently eaten "nut" in the world. Studies show that groundnut, groundnut butter and groundnut oil significantly reduce the risk of heart disease when consumed daily, similar to other nuts [2]. Groundnut oil is a vegetable oil which contains only a small proportion of non-glyceride constituents. Its fatty acid composition is complex including saturated fatty acids covering a wide range of molecular weights. Groundnut oil is excellent food oil, with good flavour and high quality with its low free fatty acid value [3]. Groundnut oil is one of the most important oil group in the world. Groundnut oil is use to lower cholesterol and prevents heart disease. It is also use to decrease appetite as an aid to weight loss. Some people use it to help prevent cancer. Recently, groundnut oil is used in ointments and medicinal oils for treating constipation. Pharmaceutical company use groundnut oil in various products they prepare for internal and external use [4-5]. Further, some studies have shown that groundnut oil contains much potassium than sodium and is a good source of calcium, phosphorus and magnesium. It also contains thiamine, vitamin E, selenium, zinc and arginine [4]. The chemical properties of oils are amongst the most important properties that determine the quality and help to describe the present condition of oils. It constitutes one of the essential components of balanced diet as good source of energy [5].

Regardless of the origin (animal, vegetable or marine), edible oils represent the highest source of energy per unit weight consumption [6]. Palm oil, soybean oil, groundnut oil, sunflower oil, olive oil, corn oil and canola oil, are some common edible oils consumed globally. Their trend of consumption is expected to show significant growth between 2016 and 2024, especially in Pacific Asia, Europe, and North America for various reasons (Persistent Market Research (https://www.prnewswire.com/news-releases/global-edible-oils-market-value-to-increase-from-us-834-bn-in-2015-to-us-1303-bn-by-2024---pmr-592605631.html).

Despite the fact that a lot of researches have been done in the past and many are still ongoing, the importance of analysing edible oils cannot be overemphasized; as major characteristics that influence their physical and chemical properties, together with their applications and uses are obtained [3]. One of such features is microbiological profile of the food. The bio-load of foods is an important criterion that determines the safety status of consuming such food, especially, as unhygienic market practices render the food products susceptible to cross-contamination. Also, the improper processing and storage conditions that groundnuts and its products are usually subjected to often result in varying degree of microbial contamination. The number and type of microbes present on the products are important indicators of their deterioration. Most commonly isolated genera of mould and bacteria include *Aspergillus*, *Penicillin* and *Fusarium*; and *Bacillus*, *Salmonella*, *Pseudomonas* and *Escherichia coli* [7]. The Enterobacteria are known to be a large group of related bacteria that are capable of food and water contamination through faecal sources. Many of their strains and species are known to be enterotoxigenic and contribute a major quota to the many diarrheal illnesses. There fore in the bid to enhance human health and secure food safety as well as public health enlightenment to food-borne illnesses, there is a need to evaluate the microbial load of all suspected contaminated products [8]. Consequently, this work was designed to assess the microbiological quality of unbranded groundnut oil, as well as their degree of susceptibility to common antibiotics.

II. MATERIALS AND METHODS

The study was conducted in the Microbiology Laboratory of Nasarawa State University, Keffi. Keffi is geographically situated on latitude 8º50ˈN and longitude 7º52ˈ E. Keffi town is about 850m above sea level and it is the North-West of Lafia, the state capital. It is 53km away from Abuja (Capital of Nigeria) in the Guinea Savannah region of Nigeria [9-10].

Sample Collection: Five (5) samples each of different unbranded groundnut oil were aseptically purchased from five (5) different spots among local sellers within Keffi Metropolis. The samples were collected in a well labelled sterile sample bottles and were aseptically transported to the laboratory for microbiological analysis, which was done within 2 hours of sample collection.

Microbiological Analysis: Microbiological analyses were conducted according to standard methods [6]. A serial dilution was performed for each of the five (5) samples by adding 1ml of the oil sample using a syringe into 9ml of water already emulsify with 10% v/v of poly surfactant 80 (Tween 80) for solubilisation of lipids to serve as the stock solution. The dilution was made up to 10^{-5}, after which 1ml of the stock of each sample was added to the 1st test tubes containing 9ml of sterile distilled water, 1ml was transferred to the 2nd test tube and the process was repeated with the 3rd, 4th and 5th test tubes. 1ml from the 4th test tubes of the diluted samples were then analysed for the microbial contents of mould and bacteria. Pour plate technique was employed that is the 1ml of each sample were poured into sterile Petri dish and the prepared and autoclaved media was poured and swirled slowly to allow for proper mixing of the sample and the media was then allowed to cool and gel. The plates were transferred to an incubator with temperature set at 37ºC for 24 hours. Nutrient agar was used for the total heterotrophic bacteria count, Salmonella/Shigella agar was used for *Salmonella* and *Shigella* while MacConkey agar was employed for the enumeration of total coliform. Similarly, pour plate technique was employ for the isolation and counts for fungi in Sabouraud Dextrose Agar incubated for 5 days at room temperature of 27±2ºC.The isolates were then determined and characterized on the bases of their cultural, morphological and biochemical characteristics as earlier described [11].

Antibiotic Susceptibility Test: The antibiotic susceptibility test was carried out as earlier described by Clinical and Laboratory Standards Institute [12]. Briefly, discrete colonies of the organism were inoculated at 37ºC for 24hrsand the overnight culture was adjusted to the turbidity equivalent to 0.5 McFarland's standard by adding 0.85% sterile normal saline to the over-night culture. The adjusted inoculums to the turbidity of 0.5 McFarland's standard were then sub-cultured on the surface of Nutrient agar and the antibiotic disc was ascetically placed at the centre of the nutrient agar plate and incubated at 37^0C for 24hrs. The diameter zones of inhibition in millimetre of the bacterial isolates recorded were then compared with the reference standard for susceptibility breakpoint of antibiotics described by Clinical and laboratory Standard Institute.

Chemical Analysis: Heavy metals such as Zn, K, Fe, Cd, Ca, Mg and Pb in the unbranded groundnut oil were also ascertained from ashing solution as described by Association of Official Analytical Chemists [13] with Atomic Absorption Spectrophotometer (AAS).

The result was then compared with standard concentrations reported for most of the metals analysed in the samples by the World Health Organisation [14] permissible limit for Zn (0.10 ppm), Cu (1.50 ppm), Cd (0.003 ppm) and Pb (0.01 ppm) in drinking water. The toxicity of these metals even at low concentrations was well documented with the view of determining whether the unbranded groundnut oils are safe or unsafe for consumption.

III. RESULTS

A total of 25 samples of unbranded groundnut oil were collected from different sellers within different locations of Keffi metropolis. The total viable bacteria count ranged between 2.1–7.2 × 10^5 cfu/ml, while the total faecal coliform count ranged from 2.2–6.2 × 10^5 cfu/ml. The *Salmonella/Shigella* count on the other hand ranged between 1.4–4.2 × 10^5 cfu/ml and the fungal count ranged between 3.6 – 8.2 × 10^5 cfu/ml (Table 1). Macroscopic identification of the fungal isolates revealed the presence of *Mucor* spp., *Aspergillus flavus, Aspergillus niger, Rhizopus* spp. and *Penicillium* spp. (Table 2). The cultural, morphological and biochemical characterization of the bacteria isolates identify the presence of *Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus* spp., *Micrococcus* spp., *E. coli* and *Salmonella* spp. (Table 3). The percentage occurrence of the bacterial isolates indicates that *Escherichia coli* (88.0%), *Salmonella* spp. (84.0%), *Pseudomonas aeruginosa* and *Staphylococcus aureus* (56.0%) were the most isolated bacteria; followed by *Micrococcus* spp. (40.0%) and *Bacillus* spp. (36.0%) (Table 4). However, the rate of occurrence of the fungal isolates indicates that *Rhizopus* spp. (88.0%) and *Mucor* spp. (84.0%) are the commonest fungal contaminants followed by *Aspergillus flavus* (56.0%), *Aspergillus niger* and *Penicillium* spp. (48.0%) (Table 5).

Anti-biogram of the bacterial isolates revealed a varying level of resistance and susceptibility to the antibiotics tested. The diameter of zone of inhibition used for this study were those defined by Johnson and Case [15], where <10mm was considered as resistant, 11–15 was considered intermediate and >16mm was considered as susceptible. All the bacterial isolates were susceptible to Septrin that was found to be resistant at 8mm. Similarly, all the isolates were very susceptible to Chloramphenicol. Also, *E. coli* and *Bacillus* spp. were slightly susceptible to Spar floxacin, but all the other bacterial isolates were found to be very susceptible to the antibiotics tested. Ciprofloxacin had 100% effect on virtually all the bacterial isolates, except *Salmonella* spp., which is highly resistant at 2mm. Nevertheless, all the bacterial isolates were resistant to Amoxicillin, except *E. coli* and *Bacillus* spp. that were only slightly susceptible with zones of 12mm each. On the other hand *Staphylococcus aureus* (20mm), *E. coli* (16mm), *P. aeruginosa* (18mm), *Bacillus* spp. (22mm) were highly susceptible to Augmentin; while *Salmonella* spp. showed some degree of susceptibility (14mm). Gentamicin has effect on all the isolates, except *S. aureus* that had slight effect at 12mm each. Perfloxacin on the other hand, was only slightly susceptible against the isolates, except *Micrococcus* spp. (18mm) and *Bacillus* spp. (20mm) that were readily susceptible. Also, Erythromycin has efficacy against all the bacteria; only *S. aureus* (14mm) has slightly susceptibility. Similarly, Streptomycin was effective against most of the isolates and only slightly susceptible to *P. aeruginosa* (14mm), *Bacillus* spp. (14mm); but *E. coli* was completely resistant to Streptomycin (Table 6).

The mineral composition of the unbranded oil samples that revealed zinc was detected from oil samples of High Court (2.55ppm) and Keffi Market (11.02ppm), but absent in the other samples. Manganese was found to be highest in CRDP (1.94ppm), followed by High Court (1.44ppm), Angwan Lambu (1.13ppm), Keffi Market (0.88ppm) and GRA (0.64ppm). The Iron contents (ppm) on the other hand were highest in oil samples from Keffi Market (13.42), High Court (9.50), CRDP (7.88), GRA (4.64) and lastly Angwan Lambu (3.47). More so, Cadmium (ppm) was found to be more predominant in oil samples from Keffi Market (0.78), compared to GRA (0.66), CRDP Area (0.60), High Court (0.42) and Angwan Lambu (0.36); while copper (ppm) was highest in Keffi Market (13.64), High Court (10.51) and Angwan Lambu (8.80), but less in CRDP Area (3.21) and GRA (3.60). Nevertheless, Lead (ppm) was detected only in oils from Angwan Lambu (2.30) and CRDP (1.02) but was absent from the other samples (Table 7).

Table 1**:** Microbial Counts of Unbranded Vegetable oil at different Locations ($\times 10^5$ cfu/ml)

Locations	TVBC	TFCC	SSC	Fungal count
High Court	3.8	2.8	1.4	8.2
Keffi Market	4.7	6.2	4.2	7.2
Angwan Lambu	3.6	3.8	2.6	6.2
CRDP Area	7.2	3.6	2.0	5.4
GRA	2.1	2.2	2.8	3.6

Table 2: Macroscopic Identification of Fungal isolates from Unbranded Groundnut Oil

Macroscopic characterization & texture	Inference Fungi
Powdery whitish surface but later turned grey with whitish reverse side and edges	*Mucor* spp.
Green fungal colony that later turned greenish-yellow or pale green	*Aspergillus flavus*
Wooly velvet, whitish in colour but later turned fungal colony with yellowish reverse side that later turned black	*Aspergillus niger*
Creamy powdery growth that later turned black	*Rhizopus* spp.
Powdery whitish surface but later turned bluish-green with whitish reverse side and edges	*Penicillium* spp.

Table 3: Cultural, Morphological and Biochemical Characteristic of Bacterial Isolates from Unbranded Groundnut oil

Cultural		PIG	M. P	G.S	Biochemical test						Probable isolates
Shape	Size (mm)				CAT	IN	V.P	MR	OX	CT	
Circular	0.4	yellowish on MSA	cocci	+	+	-	-	-	+	+	*Staph. aureus*
Circular	0.3	whitish on NA	curved rod	-	+	-	-	-	+	+	*Pseudo. aeruginosa*
Circular	4.0	whitish on NA	smooth rod	+	+	-	-	-	-	+	*Bacillus* spp.
Circular	0.5	yellow/red on NA	cocci in pairs	+	+	-	-	-	+	+	*Micrococcus* spp.
Circular	1.0	greenish on EMB/ pink on MAC	slightly curved rod	-	+	+	-	+	-	-	*E. coli*
Circular	1-2	red/black centre on SSA colourless/transparent on MAC	straight rod	-	+	-	-	+	-	+	*Salmonella* spp.

Where: MP= Morphology, GS= Grams staining, CAT= Catalase, IN= Indole, MR= Methylene red, OX= Oxidase, VP= Voges Proskauer, CT= Citrate test, + = positive, - = negative, MSA = Mannitol salt agar, EMB = Eosin methylene blue agar, NA= Nutrient agar, MAC = MacConkey agar, SSA= *Salmonella/Shigella* Agar, PIG= Pigmentation,

Table 4: Percentage Occurrence of the Bacterial Isolates at the different Locations

Location (n)	Rate of Occurrence (%)					
	P. aeruginosa	*Staph. aureus*	*Micrococcus* spp.	*E. coli*	*Bacillus* spp.	*Salmonella* spp.
High Court (5)	2(40.0)	1(20.0)	2(40.0)	5(100)	2(40.0)	4(80.0)
Keffi Market (5)	4(80.0)	4(80.0)	4(80.0)	5(100)	1(20.0)	4(80.0)
Angwan Lambu (5)	2(40.0)	3(60.0)	3(60.0)	4(80.0)	3(60.0)	5(100)
CRDP Area (5)	3(60.0)	4(80.0)	0(0.0)	5(100)	1(20.0)	3(60.0)
GRA (5)	3(60.0)	2(40.0)	1(20.0)	3(60.0)	2(40.0)	3(60.0)
Total (25)	**14(56.0)**	**14(56.0)**	**10(40.0)**	**22(88.0)**	**9(36.0)**	**19(76.0)**

Table 5: Rate of Occurrence of the Fungal Isolates (%)

Location (n)	*Aspergillus flavus*	*Aspergillus niger*	*Rhizopus* spp.	*Mucor* spp.	*Penicillium* spp.
High Court (5)	4(80.0)	4(80.0)	4(80.0)	5(100)	3(60.0)
Main Market (5)	5(100)	2(40.0)	5(100)	4(80.0)	3(60.0)
Angwan Lambu (5)	3(60.0)	3(60.0)	4(80.0)	5(100)	2(40.0)
CRDP Area (5)	2(40.0)	1(20.0)	5(100)	3(60.0)	2(40.0)
GRA (5)	2(40.0)	2(40.0)	4(80.0)	4(80.0)	2(40.0)
Total (25)	**14(56.0)**	**12(48.0)**	**22(88.0)**	**21(84.0)**	**12(48.0)**

Table 6: Anti-biogram of the Bacterial Isolates from Unbranded Groundnut Oil in Keffi Metropolis

Antibiotics	Concentration (µg)	Susceptibility of Isolates (mm)					
		S. aureus	*E. coli*	*Micrococcus* spp.	*P. aeruginosa*	*Bacillus* spp.	*Salmonella* spp.
Septrin	30	16	22	22	20	20	20
Chloramphenicol	30	18	18	22	36	24	20
Sparfloxacin	5	20	12	16	18	14	18
Ciprofloxacin	5	24	16	28	24	14	2
Amoxicillin	30	8	12	-	-	12	-
Augmentin	30	20	16	-	18	22	14
Gentamycin	10	12	28	16	18	22	22
Perfloxacin	10	14	14	18	14	20	12
Erythromycin	15	14	26	24	24	16	32
Streptomycin	10	20	-	18	14	14	18

Key: 1-10mm= Resistance, 11-15mm= Intermediate, >16mm= Susceptible, – = No inhibition

Table 7: Mineral Composition of Unbranded Groundnut oil in Part per Million (ppm)

Unbranded oil Samples location	Minerals (ppm)					
	Zn	Mn	Fe	Cd	Cu	Pb
High Court	2.55	1.44	9.50	0.42	10.51	ND
Keffi Market	11.02	0.88	13.42	0.78	13.64	ND
Angwan Lambu	ND	1.13	3.47	0.36	8.80	2.30
CRDP Area	ND	1.94	7.88	0.60	3.21	1.02
GRA	ND	0.64	4.64	0.66	3.60	ND

Key: ND= Not detected

IV. DISCUSSION

From the above results, it is evident that the bio-loads are higher than the accepted recommended load described by the International Commission for Microbiological Specification for Food [16]. It states that ready-to-eat food with plate counts between $0–13^3$ is acceptable, between $10^4 – \leq 10^5$ is tolerable and 10^6 and above is unacceptable. Preliminary isolation and identification of the microorganisms present revealed the presence of Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus spp., Salmonella spp., Micrococcus spp., E. coli, Mucor spp., Aspergillus niger, Aspergillus flavus, Penicillium spp. and Rhizopus spp. These bacteria and fungi are comparable to those isolated by some researchers [7-8], [17-18]. Escherichia coli, Salmonella spp., Pseudomonas aeruginosa and Staphylococcus aureus were the most isolated bacteria, while Rhizopus spp., Mucor spp. and Aspergillus flavus are the commonest fungal contaminants. Comparatively, a study conducted in North-West Ethiopia [19] indicated the above stated isolated microorganisms amongst those obtained in their Ethiopian samples, and that the sources of such contamination include un-sanitized measuring jug for locally produced oil, un-sanitized storage containers, exposure of oil and placement of oil with non-food substances.
Similarly, [20] noted that most edible oil produced in Ghana for human consumption is processed at the small-scale level and thus observation of the raw materials used to process such oil often show various levels of microbial contaminations that eventually get to the consumers. Also, [21] observed that these microbes are usually introduced into the vegetable oils if there is faulty handling from the manufacturers to the final consumer.

Subsequently, the isolation of harmful pathogenic microbes from the samples suggests possible potential health risks of the products that were sampled. A research by [22] had earlier stated that in microbial analysis of food, the number and type of microbes present in the food material under examination reflect quality of the food and extent of risk posed to the consumers. Anti-biogram of the bacterial isolates revealed varying levels of susceptibility/resistance to the antibiotics tested. Most of the bacterial isolates are highly resistant to Amoxicillin; however, *E. coli* was slightly susceptible (12mm). Gentamicin, Erythromycin and Chloramphenicol had the highest efficacy against all the bacteria tested. Similarly, *E. coli* was resistant to Streptomycin. Ciprofloxacin on the other hand had activity against all the isolates, except *Salmonella* spp. which is very resistant (2mm). More so, only *Micrococcus* spp. was entirely resistant to Augmentin, otherwise all the isolates were susceptible. The sensitivity pattern of the bacterial isolates to the antibiotics tested is comparable with reports of earlier researchers [23-27]. For most bacteria, there is evidence that increased usage of a particular anti-microbial correlates with increased levels of bacterial resistance [28]; perhaps this explains the high resistance to Amoxicillin by the isolates because of its common and prevalent use. Resistance to Amoxicillin is not new, as [29] had observed such effect earlier. The high susceptibility of the isolates to Gentamicin and Chloramphenicol observed in this study might be due to their requirement for parenteral administration which hinder their misuse and abuse considered to be the major source of microbial resistance to conventional antibiotics as observed by [30]. From the result obtained for the mineral contents analysis, all the samples showed detectable levels of Zn (2.55–11.02ppm), Mn (0.64–1.44ppm), Fe (4.64–13.42ppm), Cu (3.21–13.64ppm), Cd (0.36–0.78ppm) and Pb (1.02–2.30ppm). The mineral contents obtained in this study are comparable to that reported by [3] and [31]. The concentrations reported for most of the metals analysed in the samples exceeded the WHO (2008) permissible limit for Zn (0.10ppm), Cu (1.50ppm), Cd (0.003ppm) and Pb (0.01ppm) in drinking water. Determination of heavy metals in edible seed oil is of importance, as heavy metals are useful micronutrients for plants, humans and animals but become toxic for them when their concentration exceeds a limit [32]. The toxicity of these metals even at low concentrations is well documented, thereby making these edible seed oils unsafe for consumption.

V. CONCLUSION

The microbiological quality assessment of unbranded groundnut oils sold in Keffi revealed high bio-load that exceed the maximum limits set by the International Commission for Microbiological Specification of Foods (ICMSF), Food and Agricultural Organization of the United Nations (FAO) and the National Agency for Food Drug Administration and Control of Nigeria (NAFDAC). The presence of enteric bacteria is evidence of faecal-contamination which may result from contaminated handling equipments, and general processing. Also, the presence of *Aspergillus* species such as *A. flavus* and *A. niger, Mucor* spp., *Rhizopus* spp. and *Penicillium* spp. in the groundnut oil samples pose a toxicological threat to the consumers since majority of the strains of these

fungal species are toxigenic. It is therefore, recommended that the appropriate quality standards should be adopted in the production of these unbranded vegetable oils. Also, indiscriminate prescription and use of antibiotics should be avoided so as to checkmate the rising resistant of microbes to antibiotics.

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